

pixelated detectors in imaging system 70 in what might be referred to as a "stereo or three-dimensional configuration" allows flexibility in the configuration of each leg of the system, including parameters such as the relative pixelated readout rates, axial orientations, inclinations, focal plane positions, and magnification. Multiple beads or other objects may be imaged onto each detector simultaneously in the vertical direction. Since the beads may move in synchronicity with the signal on the pixelated detector, no gate or shutter is required to prevent blurring of the image. As previously noted, the present invention can use a pulsed or CW light source without need for a trigger mechanism to time a pulse coincident with particle arrival in the FOV.

Also illustrated in FIGURE 4 are several exemplary positions for light sources, which are useful for different purposes in connection with the imaging system illustrated therein. In connection with pixelated detector 44a, light source 62 provides illumination of bead 24 from a direction so that absorption characteristics of the bead can be determined from the image produced on the pixelated detector. At the same time, light provided by light source 62 that is scattered from bead 24 can be used to produce a scatter image and spectrally dispersed images on pixelated detector 44b. A light source 74 can be employed to produce spectrally dispersed and scattered images on both pixelated detectors 44a and 44b. If light sources 62 and 72 are of different wavelengths and an appropriate filter is provided to block the wavelength from the light source aligned with the optical axis of the respective collections lenses 32, these two light sources can be used for producing scattered light from the bead. For example, suppose light source 72 produces light of a wavelength A that scatters from bead 24 and is directed toward pixelated detector 44a. By including a filter (not shown) that blocks wavelength B produced by light source 62, the light at wavelength B will not directly affect the images produced on pixelated detector 44a. Similarly, the light from light source 72 would be blocked with an appropriate filter (not shown), so that it does not interfere with the imaging of light produced by light source 62 that is scattered from bead 24 onto pixelated detector 44b.

Epi light source 66 is also illustrated for use in producing images on pixelated detector 44a in conjunction with partial reflector 68. Light source 64 can be used to generate reflected light to produce images on pixelated detector 44a, while scattered light from this source is directed toward pixelated detector 44b. These and other possible locations of light sources will be apparent to those of ordinary skill in the art, as appropriate, for providing the incident light on the bead needed to achieve imaging, depending upon the particular application and information about the bead that is desired.

FIGURE 5 illustrates a detector used in the present invention, with a spectral resolution of approximately 10 nm per pixel and a spatial resolution of approximately 0.5 microns. In the following discussion of FIGURES 5-8, the operation of the imaging system is directed toward the identification of fluorescence *in situ* hybridization (FISH) probes bound to specific DNA with cells. However, those skilled in the art will appreciate that the same method and apparatus applies to the spectral and spatial information resulting from the imaging of reporters associated with beads. With respect to the following discussion, a cell and nucleus can be considered to be equivalent to a bead, and FISH probes can be considered to be equivalent to reporters associated with or bound to a bead.

FIGURE 5 illustrates how the imaging system that is usable to image reporter beads is used to image a cell 140 having a nucleus 142 in which are disposed two FISH probes 144a and 144b having the same emission spectrum. As noted above, in a bead analysis, the FISH spots may be considered analogous to reporters. In FIGURE 5, the emission spectrum 146 of FISH probes 144a and 144b is approximately 10 nm in width, such as would be produced by "quantum dots" or a narrowband fluorescent dye. The optical convolution of the narrow bandwidth spectrum results in minimal blurring of FISH spots 148a and 148b, enabling them to be readily resolved on detector 44.

In FIGURE 6, a cell 150 is illustrated having a nucleus 152 in which are disposed FISH probes 154 and 156 having different emission spectra. Each of the emission spectra of FISH probes 154 and 156 are relatively narrow, such as the emission spectra from quantum dots, as indicated by wavebands 158 and 160, and therefore, as in FIGURE 5, minimal blurring occurs in FISH spots 162 and 164. Furthermore, the spectral dispersion of the present invention, which maps wavelength into lateral position on TDI detector 44, produces a relatively wide physical displacement of FISH spots 162 and 164, despite the close proximity of FISH probes 154 and 156 in the cell. Taken together, FIGURES 5 and 6 illustrate how the present invention discriminates FISH probes of the same or different color, thereby enabling the simultaneous enumeration of numerous genetic traits. FIGURE 10 illustrates how a cell and FISH spots contained therein will be imaged by the present invention upon a pixelated detector.

FIGURES 7 and 8 illustrate that the present invention can also be used with light of wider spectral bandwidth. In this case, an additional signal processing step is performed to correct for lateral blurring due to the wide emission spectra. Note that this involves a deconvolution step which requires the use of a pixelated detector that is a TDI detector. In FIGURE 7, a cell 140 having a nucleus 142 is shown, and FISH probes 170a and 170b having a common emission spectrum are disposed in the

nucleus. FISH probes 170a and 170b are characterized by producing a relatively wide emission spectrum 172. When optically convolved by the spectral dispersion provided by the present invention, FISH spots 174a and 174b are produced on a TDI detector 44a, but their images are laterally blurred across pixelated detector 44, as a  
5 result of their relatively wide emission spectrum. To more clearly resolve the separation of FISH spots probes 174a and 174b, a deconvolution is carried out on the signal produced by TDI detector 44a, with the known FISH emission spectrum, thereby producing accurate FISH spot representations 178a and 178b on a display 176. The deconvolution step enhances the ability to enumerate the number of FISH spots. Note  
10 that the deconvolution step requires the use of a pixelated detector that is a TDI detector.

FIGURE 8 illustrates a corresponding relationship between FISH probes 180 and 182, which are disposed within a nucleus 152 of a cell 150. FISH probes 180 and 182 are characterized by each producing relatively wide band emission spectra 184 and 186, as shown in the Figure. Optical convolution of the fluorescence emitted by  
15 the FISH probes, which are spectrally dispersed, produces FISH spots 188 and 190 on TDI detector 44. Again, by deconvolving the known FISH emission spectra with the signal produced by TDI detector 44, the corresponding images shown on display 176 of FISH spots 192 and 194 are recovered. Again, the spectral dispersion of the present  
20 invention, which maps wavelength into lateral position on TDI detector 44, produces a relatively wide physical displacement of FISH spots 192 and 194, despite the close proximity of FISH probes 180 and 182 in the cell. In this manner, it is possible to resolve these images of FISH spots produced by FISH probes having different and relatively wide emission spectra.

A system 230 for analyzing the signal produced by TDI detector 44a and performing the deconvolution steps described above is illustrated in FIGURE 9. In this figure, the signal from TDI detector 44a is applied to an amplifier 232, which buffers the signal and amplifies it to achieve a level required by an analog-to-digital (A/D) converter 234. This A/D converter converts the analog signal from amplifier 232 into a  
30 digital signal that is input into a line buffer 236. Line buffer 236 temporarily stores the digital signal until it can be processed by a CPU 238. To carry out the deconvolution noted above, a spectral buffer 240 is loaded with the known emission spectrum for each of the FISH probes being used so that their emission spectra can be deconvolved with the signal stored in line buffer 236. CPU 238 is a high speed processor programmed to  
35 carry out the deconvolution and other analysis procedures, enabling the identification of desired characteristics or parameters of the object being imaged. The output from